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(54) Title: ANTICANCER COMPOUNDS

(57) Abstract

Compounds comprising an antioestrogenic moiety linked to an antitumour moiety by means of a spacer group characterised in that the spacer group is a minimum of 10 atoms in length excluding any atoms from the antioestrogenic moiety or the antitumour moiety which contribute to the linkage.

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ANTI-CANCER COMPOUNDS

FIELD OF THE INVENTION

This invention relates to novel compounds and their use as chemotherapeutic agents.

BACKGROUND TO THE INVENTION

The number of chemotherapy agents of use in the treatment of malignant tumours has expanded rapidly. The diversity of tumours has led to the development of a wide range of chemotherapeutic agents of varying specificity. For example many agents designed to be active at steroid hormone receptors are cytotoxic to cells not bearing those receptors. Similarly antitumour agents such as anthracyclines are often active at non-tumourous cells. It is the lack of specificity of many such agents that leads to the undesirable side effects associated with chemotherapy. These side effects often result in a patient taking a combination of chemotherapeutic agents together with other drugs to minimise the side effects. There is therefore a need for more specific chemotherapeutic agents.

SUMMARY OF INVENTION

The present invention provides novel compounds of use as chemotherapeutic agents that seek to overcome the drawbacks of prior art chemotherapeutic agents, and provide an improved specificity of chemotherapeutic activity.

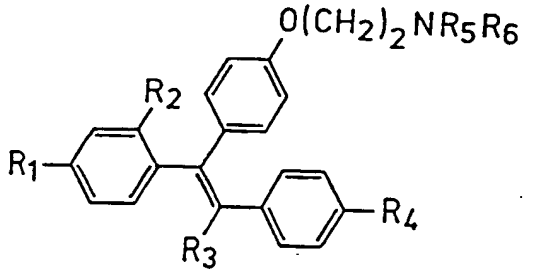
Accordingly, the invention relates to compounds comprising an antioestrogenic moiety linked to an antitumour moiety by means of a spacer group, characterised in that the spacer group is a minimum of 10 atoms in length excluding any atoms from the anti-oestrogenic moiety or the antitumour moiety which contribute to the linkage.

The term "antioestrogenic moiety" is used herein to refer to a chemical that has activity at an oestrogen receptor that results in an inhibition of protein biosynthesis.

The term "antitumour agent" is used herein to refer to a chemical having direct or indirect antimitotic activity, other

than by activity at an oestrogen receptor. Such an agent may be an alkylating agent, an intercalating agent or an antimetabolite, e.g. an antifolate, etc. A wide range of such agents is discussed in general in The Oxford Textbook of Medicine, 2nd Edition, pages 4.131 to 4.139 (Oxford University Press).

More particularly the invention relates to compounds wherein the antioestrogenic moiety is selected from the group consisting of compounds of general formula (I)



(I)

wherein R_1 is hydrogen or hydroxy, R_2 is hydrogen or C_{1-4} alkyl, R_3 is unsubstituted aliphatic C_{1-4} alkyl or aliphatic C_{1-4} alkyl substituted by one or more halogen, nitro, amino, aldehyde, keto, hydrazino or alcohol groups, R_4 is halogeno and R_5 and R_6 are hydrogen or aliphatic C_{1-4} alkyl, R_5 and R_6 being the same or different; or a 2,3-diphenylindole, 2-benzoyl-3-phenylthiophene, an acetoxy-substituted triarylethene, a 1,2-diphenylethane or a 2-phenylindene, and the antitumour moiety is selected from the group consisting of anthracycline drugs such as the compounds doxorubicin, daunorubicin, epirubicin, idarubicin and also mitoxantrone and the compounds methotrexate, aclacinomycin A, DUP-941, cyclophosphamide and ifosphamide.

It has been found that when an antioestrogenic agent is linked to an antitumour agent by means of a spacer, there is an increase in the specificity of the antitumour moiety. The length of the spacer is important in contributing to this effect. It is this observation that lies at the heart of the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

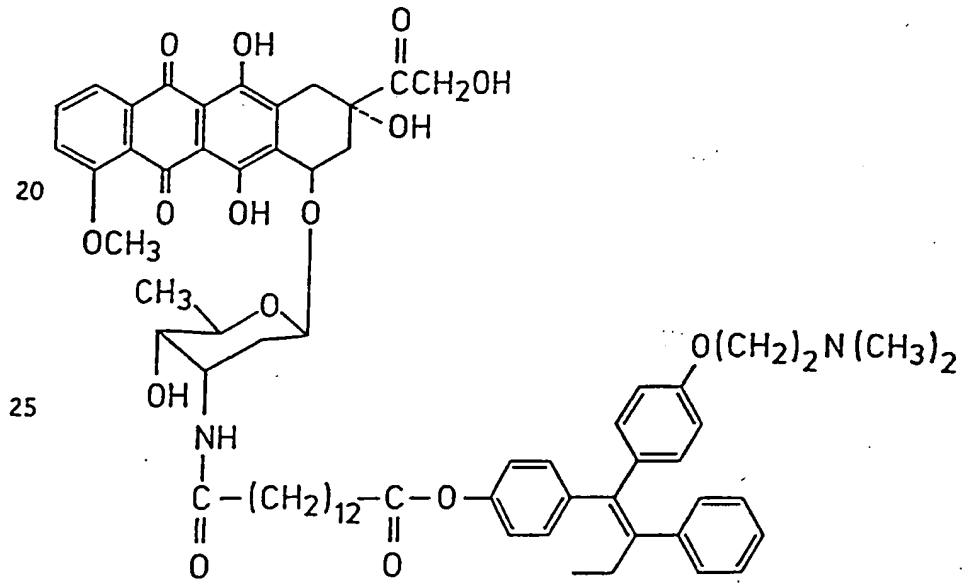
Preferred compounds of the present invention are those wherein the antioestrogenic agent is tamoxifen, 2-methyl-4-hydroxytamoxifen, 4-iodotamoxifen, 4-fluorotamoxifen or 4-hydroxytamoxifen and the antitumour agent is doxorubicin, methotrexate or mitoxantrone. Most preferably the antioestrogenic moiety is tamoxifen or 4-hydroxytamoxifen.

Any chemical group can be used as a spacer group to link the antioestrogenic and antitumour agents. The length of the spacer group must be a minimum of 10 atoms in length. This excludes any atoms contributing to the linkage which derive from the antitumour moiety or the antioestrogenic moiety. The maximum length of the spacer group is not as important. The length of the spacer is preferably a maximum of 22 or 24 atoms in length and more preferably of between 12 and 18 atoms in length, preferably between 13 or 16 atoms in length and more preferably 14 atoms in length. The spacer group may be made up from a spacer moiety and separate linker moieties at either end of the spacer moiety that chemically link the spacer moiety to the antioestrogenic and antitumour moieties. In this case, where there are linker moieties, the number of atoms in the linker moiety or moieties contributes to the number of atoms when calculating the length of the spacer group. Suitable spacer moieties include Michael Addition spacers, for example, glutaraldehyde amidinium, hydrocarbons, oligopeptides and polypeptides including peptide polymers, oligosaccharides and polysaccharides, for example, dextran or glycogen, polymers such as polyalkylcyanoacrylates and proteins such as bovine or human albumin, immunoglobulins, monoclonal antibodies, ferritin, catalase or superoxide dismutase.

Suitable linker moieties include the following chemical groups: diazonium, hydrazone, hemiacetal, hemiketal, acetal, ketal, hemisuccinate, carboxymethylamine, oxazolidine, sulphydryl, cystamine, carbon-ester, phosphate-ester, thioester, thioether, imine (Schiff base), ether or N-hydroxy succinamide

ester. The linker moiety can be the same or different at either end of the spacer. Preferably, however, the spacer group does not require separate linker moieties and links directly to the active moieties, for example being a dicarboxylic acid, a polyamino acid or a polysaccharide. Preferred examples of a dicarboxylic acid are those of the formula $HOOC(CH_2)_nCOOH$ where n is from 8 to 20 (providing a spacer group from 10 to 22 atoms), preferably 10-16 and more preferably 12, of a polyamino acid is polyglutamic acid or polylysine and of a polysaccharide is dextran.

Preferably the spacer group makes an ester or amide bond linkage with the antioestrogenic and antitumour agent. Most preferable is a spacer group that is a dicarboxylic acid of the formula $HOOC(CH_2)_nCOOH$ where n is 8 or 12. An example of a preferred compound of the invention is that of formula (II)



(II)

In this compound the antioestrogenic moiety is 4-hydroxytamoxifen and the antitumour agent is doxorubicin. The spacer group is a dicarboxylic acid of the formula

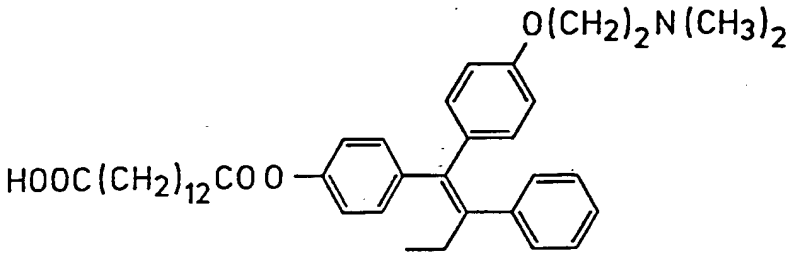
$HOOC(CH_2)_{12}COOH$ making an ester bond linkage with the 4-hydroxytamoxifen and an amide bond linkage with doxorubicin.

A further aspect of the present invention includes compounds that are the biological breakdown products of compounds comprising an antioestrogenic moiety linked to an antitumour moiety, with the proviso that the biological breakdown product includes at least part of the spacer group.

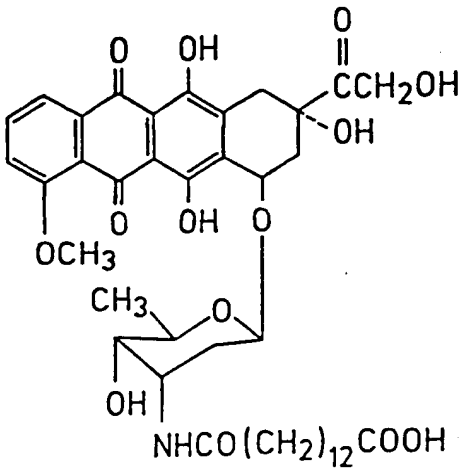
The term "biological breakdown product" is used herein to refer to any product that may result from the hydrolysis, spontaneous or enzymatic breakdown of a compound of the present invention.

Thus, the present invention further relates to compounds comprising an antioestrogenic moiety or an antitumour moiety linked to all or part of a spacer group.

By way of example with reference to the compound of formula (II), biological breakdown products may include the compounds of formulae (III) and (IV)



(III)



(IV)

All the compounds of the present invention are believed to be novel.

The invention further includes the use of these compounds in therapy, particularly in the treatment of a cancer.

The compounds of the present invention may be formulated with a physiologically acceptable diluent or carrier for use as pharmaceuticals for both veterinary, for example in mammals, and particularly human use by a variety of methods. For instance, they may be applied as a composition incorporating a liquid diluent or carrier, for example an aqueous or oily solution, suspension or emulsion, which may often be employed in injectable form for parenteral administration and therefore may conveniently be sterile and pyrogen free. Oral administration may also be used and although compositions for this purpose may incorporate a liquid diluent or carrier, it is more usual to use a solid, for example a conventional solid carrier material such as starch, lactose, dextrin or magnesium stearate. Such solid compositions may take the form of powders but are more conveniently of a

formed type, for example as tablets, cachets, or capsules (including spansules). Alternative, more specialized types of formulation include liposomes and nanoparticles.

Other types of administration than by injection or through the oral route which are of use in both human and veterinary contexts include the use of suppositories or pessaries. Another form of pharmaceutical composition is one for buccal or nasal administration. Other formulations for topical administration include lotions, ointments, creams, gels and sprays.

Compositions may be formulated in unit dosage form, i.e. in the form of discrete portions containing a unit dose, or a multiple or sub-unit of a unit dose.

Whilst the dosage of the compound used will vary according to the activity of the particular compound and the condition being treated, it may be stated by way of guidance that a dosage selected in the range from 10 to 500 mg/kg per body weight per day.

All the antioestrogenic and antitumour moieties of use in forming the compounds of the present invention must possess an accessible group capable of linking to the spacer group, e.g. the hydroxy group of 4-hydroxytamoxifen, the amino group of the amino sugar daunosamine of doxorubicin. For the antioestrogen moiety tamoxifen and others like it not having an immediately accessible group for linkage to a spacer, the skilled man will be capable of identifying a "non-essential" side group, i.e. a side chain that may be replaced without detracting from the biological activity of the moiety concerned, for example the ethyl group of tamoxifen, that can be modified to provide an accessible group by replacement with an aminomethyl group providing the possibility of an amide linkage bond between tamoxifen and a spacer.

The invention will now be demonstrated in the following Examples.

ABBREVIATIONS USED

DoxoTam n : A compound according to the invention comprising doxorubicin (Doxo) and 4-hydroxytamoxifen (Tam)

linked by an $HOOC(CH_2)_nCOOH$ spacer group, where n is 2, 8 or 12.

Tam n : A 4-hydroxytamoxifen derivative linked to $HOOC(CH_2)_nCOOH$ where n is 2, 8 or 12.

Doxorester n : A doxorubicin derivative linked to $HOOC(CH_2)_nCOOH$ where n is 2, 8 or 12.

EXAMPLE 1

Synthesis of Tamoxifen and Doxorubicin esters

I. SYNTHESIS OF TAMOXIFEN ESTERS

Z-4-Hydroxytamoxifen was prepared as described in McCague R. (1986) J. Chem. Res. (S) 58-59 and J. Chem. Res. (M) 0771-0793.

(a) Synthesis of Z-4-Hydroxytamoxifen Succinoyl Ester (Tam 2)

Z-4-Hydroxytamoxifen (5 mg, 12.9 µmol) was dissolved in pyridine (10 ml) and succinyl chloride (4 µl, 37.9 µmol) was added and the mixture was stirred at room temperature for 2 hours. The pyridine was evaporated using compressed air and the residual solid redissolved in phosphate buffer pH 7.0. The compound was separated by column chromatography (2.5 cm x 15 cm, silica gel column, eluted with 25% ethyl acetate in hexane, triethylamine, glacial acetic acid (100:5:5)), the resulting white solid (approximately 3 mg, 6.16 µmol, 47.8%) had an Rf of 0.78 slightly less than Z-4-hydroxytamoxifen (0.83) using normal phase TLC with a mobile phase of acetone/triethylamine (20:1).

Analysis: Mpt : decomposition at approximately 185°C.

IR : 3300-3800 cm^{-1} (COOH), 1660 cm^{-1} (C=O), 1020-1340 cm^{-1} (ethoxy), 3000-3100 cm^{-1} (C-H, aromatic), 1600 cm^{-1} (C=C, aromatic).

^1H NMR ($CDCl_3$) : (270 mHz) 0.97 (3H, t, CH_2CH_3), 1.13 (EtOH), 2.50 (2H, q, CH_2CH_3), 2.74 (2H, t, OCH_2CH_2N), 2.82 (6H, s, NMe_2), 2.88 (2H, d, CH_2CH_2COOH), 2.94 (2H, d, CH_2CH_2COOH), 3.01 (EtOH), 3.88 (2H, q, OCH_2CH_2N), 6.78 (2H, d, meta to OCH_2CH_2N), 7.00 (2H, d, ortho to OCH_2CH_2N), 7.07-7.59 (remaining ArH), 10.10 (1H, s, CH_2CH_2COOH, D_2O exchangeable).

Mass Spectrum: m/e (relative intensity) 474 (40, $C_{30}H_{30}NO_3$), 72 (15, $C_4H_{10}N$), 58 (51, C_3H_8N).

Elemental Analysis: Found C, 71.45%; H, 8.06%; N, 4.90%, calculated for $C_{30}H_{31}NO_5$ C, 76.59%; H, 6.60%; N, 2.98%.

(b) Synthesis of Z-4-Hydroxytamoxifen Sebacoyl Ester (Tam 8)

Z-4-Hydroxytamoxifen (50 mg, 129 µmol) was dissolved in pyridine (10 ml) and sebacoyl chloride (30 µl, 141 µmol) added. The mixture was stirred at room temperature for 8 hours, then the pyridine was removed using compressed air. The residual solid was redissolved in 25% ethyl acetate in hexane and separated using normal phase column chromatography (2.5 cm x 15 cm, silica gel for flash chromatography, eluted with 25% ethyl acetate in hexane, triethylamine, glacial acetic acid (100:5:5)). The ester (30 mg, 52.7 µmol, 40.8% has a retention factor (Rf 0.10) less than Z-4-hydroxytamoxifen (Rf 0.44) and so eluted after the parent compound.

Analysis: Mpt : 46-48°C.

IR : 3300-3800 cm^{-1} (COOH), 1660 cm^{-1} (C=O), 1020-1340 cm^{-1} (ethoxy).

^1H NMR ($CDCl_3$) : (270 mHz) 0.98 (3H, t, CH_2CH_3), 1.21 (EtOH), 2.17 (6H, s, NMe_2), 2.28 (2H, s, $(CH_2)_8$), 2.52 (2H, q, CH_2CH_3), 2.73 (2H, t, OCH_2CH_2N), 2.49-2.73 (m, $(CH_2)_8$), 3.87 (2H, q, OCH_2CH_2N), 6.75 (2H, d, meta to OCH_2CH_2N), 6.97 (2H, d, ortho to OCH_2CH_2N), 7.16-7.57 (remaining ArH), 9.50 (1H, s, CH_2CH_2COOH, D_2O exchangeable).

Mass Spectrum: m/e (relative intensity) 554 (1, M), 496 (1, $C_{33}H_{36}NO_4$), 387 (1, $C_{26}H_{29}NO_2$), 58 (105, C_3H_6N).

Elemental Analysis: Found C, 86.64%; H, 10.50%; N, 5.05%, calculated for $C_{36}H_{43}NO_5$ C, 77.98%; H, 7.76%; N, 2.53%.

(c) Synthesis of Z-4-Hydroxytamoxifen Dodecanedioyl Ester (Tam 12)

Z-4-Hydroxytamoxifen (50 mg, 129 µmol) was dissolved in pyridine (10 ml) and dodecanedioyl chloride (30 µl, 120 µmol) added. The mixture was stirred at room temperature for 8 hours. The pyridine was then removed using compressed air. The residual solid was redissolved in 25% ethyl acetate in hexane and separated using normal phase column chromatography (2.5 cm x 15 cm, silica gel for flash chromatography, eluted with 25% ethyl acetate in hexane, triethylamine, glacial acetic acid

(100:5:5)). The ester (35 mg, 55.8 mol, 48.8%) has a retention factor (Rf 0.12) less than Z-4-hydroxytamoxifen (Rf 0.44) and so eluted after the parent compound.

Analysis: Mpt : 49-51°C.

IR : 3300-3800 cm^{-1} (COOH), 1660 cm^{-1} (C=O), 1020-1340 cm^{-1} (ethoxy).

^1H NMR ($CDCl_3$) : (270 mHz) 0.97 (3H, t, CH_2CH_3), 1.12 (EtOH), 2.23 (6H, s, NMe_2), 2.33 (2H, s, $(CH_2)_{12}$), 2.48 (2H, t, CH_2CH), 2.73 (2H, t, OCH_2CH_2N), 2.50-2.84 (2H, m, $(CH_2)_{12}$), 3.87 (2H, q, OCH_2CH_2N), 6.72 (2H, d, meta to OCH_2CH_2N), 6.99 (2H, d, ortho to OCH_2CH_2N), 7.06-7.57 (remaining ArH), 7.88 (1H, s, CH_2CH_2COOH, D_2O exchangeable).

Mass Spectrum: m/e (relative intensity) 628 (1, M), 570 (1, $C_{33}H_{37}NO_5$), 387 (1, $C_{26}H_{29}NO_2$), 58 (105, C_3H_6N).

Elemental Analysis: Found C, 72.40%; H, 9.32%; N, 3.84%, calculated for $C_{40}H_{53}NO_5$ C, 76.40%; H, 8.40%; N, 2.30%.

II. SYNTHESIS OF DOXORUBICIN ESTERS

(a) Synthesis of Doxorubicin Succinoyl Ester (Doxorester 2)

Doxorubicin HCl (8 mg, 12.8 µmol) was dissolved in dichloromethane (10 ml). Succinyl chloride (5 µl, 47.35 µmol) was added with 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide (ECDI) (14 mg, 70 µmol). The mixture was stirred at room temperature for 4 hours. Any unreacted doxorubicin was removed by extraction with phosphate buffer, pH 5.0 (at which pH the unreacted doxorubicin will be ionised). The dichloromethane was evaporated to give a red solid which was then recrystallised from chloroform. The compound (5 mg, 8.5 µmol, 66.7%) has an Rf of 0.93 compared to an Rf of 0.46 for doxorubicin using normal phase TLC with a mobile phase of chloroform/methanol/triethylamine (70:10:1).

Analysis: Mpt : 210-212°C.

IR : 3000 cm^{-1} (alkyl), 1700-1750 cm^{-1} (C=O), 3300-3800 cm^{-1} (COOH).

^1H NMR ($CDCl_3$) : (270 mHz) 1.12 (3H, d, CH_3, C5'), 1.91 (2H, s, C2'), 2.20 (2H, s, C9), 2.67 (2H, s, C7), 1.80/2.40 (2H, d, $(CH_2)_2$),
3.20 (1H, s, CH, C3'), 3.40 (1H, s, CH, C4'), 3.70 (3H, s, CH_3O),
3.82 (1H, q, CH, C5'), 4.26 (2H, s, C14), 4.54 (1H, s, C10),
4.99 (1H, s, C1'), 7.23 (1H, t, C3), 7.26 (2H, d, C2, C4),
13.08/13.85 (2H, s, OH, (C6, C11) D_2O exchangeable),
9.37 (1H, S, COOH, D_2O exchangeable).
Mass Spectrum: m/e (relative intensity) 627 (1, M),
158 (19, $C_6H_8NO_4$), 129 (35, $C_5H_7NO_3$), 115 (51, $C_4H_5NO_3$),
101 (5, $C_4H_5O_3$).
Elemental Analysis: Found C, 60.21%; H, 4.80%; N, 2.45%,
calculated for $C_{31}H_{31}NO_{14}$ C, 59.33%; H, 4.94%; N, 2.23%.

(b) Synthesis of Doxorubicin Sebacoyl Ester (Doxorester 8)

Doxorubicin HCl (8 mg, 12.8 µmol) was dissolved in dichloromethane (10 ml). Sebacoyl chloride (5 µl, 229 µmol) was added with 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide (ECDI) (14 mg, 70 µmol). The mixture was stirred at room temperature for 4 hours. Any unreacted doxorubicin was removed by extraction with phosphate buffer, pH 5.0 (at which the unreacted doxorubicin will be ionised). The dichloromethane was evaporated to give a red solid, which was recrystallised from chloroform. The compound (4 mg, 5.52 µmol, 43.1%) has an Rf of 0.64 compared to an Rf of 0.46
for doxorubicin using normal phase TLC with a mobile phase of chloroform/methanol/triethylamine (70:10:1).
Analysis: Mpt : 214-215°C.
IR : 3000 cm^{-1} (alkyl), 1700-1750 cm^{-1} (C=O),
3300-3800 cm^{-1} (COOH).
^1H NMR ($CDCl_3$) : (270 mHz) 1.18 (3H, d, CH_3, C5'),
1.61 (2H, s, C2'), 2.17 (2H, s, C9), 2.56 (2H, s, C7),
2.10-2.49 (2H, m, $(CH_2)_8$), 3.23 (1H, s, CH, C3'),
3.49 (1H, s, CH, C4'), 3.66 (3H, s, CH_3O), 3.85 (1H, q, CH, C5'),
4.11 (2H, s, C14), 4.81 (1H, s, C10), 5.07 (1H, s, C1'),

6.85 (1H, t, C3), 7.26 (2H, d, C2, C4), 13.85/13.08 (2H, s, OH, (C6, C11) D_2O exchangeable), 9.19 (1H, s, COOH, D_2O exchangeable).
Mass Spectrum: m/e (relative intensity) 711 (1, M), 158 (24, $C_6H_8NO_4$), 129 (43, $C_5H_7NO_3$), 115 (43, $C_4H_5NO_3$), 101 (9, $C_4H_5O_3$).
Elemental Analysis: Found C, 62.31%; H, 6.21%; N, 1.82%, calculated for $C_{37}H_{43}NO_{14}$ C, 62.45%; H, 6.05%; N, 1.97%.

(c) Synthesis of Doxorubicin Dodecanedioyl Ester (Doxorester 12)

Doxorubicin HCl (8 mg, 12.8 µmol) was dissolved in dichloromethane (10 ml). Dodecanedioyl chloride (5 µl, 200 µmol) was added with 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide (ECDI) (14 mg, 70 µmol). The mixture was stirred at room temperature for 4 hours. Any unreacted doxorubicin was removed by extraction with phosphate buffer, pH 5.0. The dichloromethane was evaporated to give a red solid, which was recrystallised from chloroform. The compound (6 mg, 7.68 µmol, 60.0%) has an Rf of 0.63
compared to an Rf of 0.46 for doxorubicin using normal phase TLC with a mobile phase of chloroform/methanol/triethylamine (70:10:1).
Analysis: Mpt : 230-235°C.
IR : 3000 cm^{-1} (alkyl), 1700-1750 cm^{-1} (C=O), 3300-3800 cm^{-1} (COOH).
^1H NMR ($CDCl_3$) : (270 mHz) 1.18 (3H, d, CH_3, C5'), 1.61 (2H, s, C2'), 2.20 (2H, s, C9), 2.57 (2H, s, C9), 1.87-3.17 (2H, m, $(CH_2)_{12}$), 3.33 (1H, s, CH, C3'), 3.49 (1H, s, CH, C4'), 3.66 (3H, s, CH_3O), 3.79 (1H, q, CH, C5'), 4.10 (2H, s, C14), 4.97 (1H, s, C10), 5.25 (1H, s, C1'), 6.83 (1H, t, C3), 7.26 (2H, d, C2, C4), 13.85/13.08 (2H, s, OH, (C6, C11) D_2O exchangeable), 9.20 (1H, s, COOH, D_2O exchangeable).
Mass Spectrum: m/e (relative intensity) 767 (1, M), 158 (24, $C_6H_8NO_4$), 129 (43, $C_5H_7NO_3$), 115 (43, $C_4H_5NO_3$), 101 (9, $C_4H_5O_3$).
Elemental Analysis: Found C, 63.71%; H, 6.53%; N, 1.65%, calculated for $C_{41}H_{51}NO_{14}$ C, 64.15%; H, 5.34%; N, 2.58%.

EXAMPLE 2

Synthesis of Z-4-Hydroxytamoxifen - Doxorubicin Prodrugs

I. SYNTHESIS OF Z-4-HYDROXYTAMOXIFEN SUCCINOYL ESTER - DOXORUBICIN PRODRUG (DOXOTAM 2)

Z-4-Hydroxytamoxifen succinoyl ester (Tam 2, 5 mg, 10.2 µmol) was dissolved in phosphate buffer pH 7.0 (10 ml). Doxorubicin HCl (5 mg, 8 µmol) in 3 ml distilled water was added with 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide (ECDI) (14 mg, 70 µmol). The mixture was stirred at room temperature for 4 hours. The product was removed by extraction from phosphate buffer, pH 5.0 (at which unreacted doxorubicin will be ionised) into dichloromethane which was evaporated to give a pink solid (4 mg, 3.95 µmol, 49.4%) which was recrystallised from methanol. HPLC studies using 35% acetonitrile, 0.02M NaH_2PO_4, 0.05% triethylamine, pH 4.0 on a C_{18} reverse phase column (10 cm) showed a peak with a retention time of about 2.30 minutes. After acid hydrolysis of the product (gentle heat at pH 4.0 for 10 minutes) there was a peak at approximately 6.69 minutes corresponding to doxorubicin. Normal phase TLC with a mobile phase of acetone/triethylamine (20:1) also showed a component in the acid hydrolysed product corresponding to 4-hydroxytamoxifen.

Analysis: Mpt : 162-165°C.

IR : 1612 cm^{-1}, 1587 cm^{-1}, 1282 cm^{-1}, 1204 cm^{-1}, 1010 cm^{-1}, 990 cm^{-1} (Doxorubicin).

^1H NMR ($CDCl_3$) : (270 mHz) 0.89 (3H, t,m CH_2CH_3), 1.15 (3H, d, CH3, C5'), 1.25 (s, EtOH), 1.91 (2H, s, C2'), 2.03 (6H, s, NMe_2), 2.20 (2H, s, C9), 1.80 (2H, d, CH_2CH_2), 2.22 (2H, d, CH_2CH_2), 2.50 (2H, q, CH_2CH_3), 2.74 (2H, t, OCH_2CH_2N), 2.67 (2H, s, C7), 3.32 (1H, s, CH, 3'), 3.34 (1H, s, CH, 4'), 3.80 (2H, q, OCH_2CH_2N), 3.60 (3H, s, CH_3O), 3.82 (1H, C5'), 4.24 (2H, C14), 4.39 (1H, C10), 4.99 (1H, s, C1'), 6.89 (2H, d, meta to OCH_2CH_2N), 7.18-7.49 remaining H,

(underlining indicates signals due to doxorubicin).

Mass Spectrum: m/e (relative intensity) 629 ($C_{31}H_{35}NO_{13}$,51), 570 ($C_{28}H_{30}NO_{12}$,14), 558 ($C_{34}H_{42}N_2O_5$,23), 542 ($C_{27}H_{30}NO_{11}$,2), 430 ($C_{28}H_{32}NO_2$,34), 402 ($C_{26}H_{28}NO_2$,22), 356 ($C_{19}H_{16}O_7$,5), 282 ($C_{19}H_{24}NO$,3).

Elemental Analysis: Found C, 66.40%; H, 5.46%; N, 2.52%, calculated for $C_{57}H_{60}N_2O_{15}$ C, 67.50%; H, 5.92%; N, 2.76%.

II. SYNTHESIS OF Z-4-HYDROXYTAMOXIFEN SEBACOYL ESTER - DOXORUBICIN PRODRUG (DOXOTAM 8)

Z-4-Hydroxytamoxifen sebacoyl ester (Tam 8, 5 mg, 4.6 µmol) was dissolved in dichloromethane (10 ml). Doxorubicin HCl (5 mg, 8 µmol) was added with N,N' dicyclohexylcarbodiimide (10 mg, 48 µmol). The mixture was stirred at room temperature for 4 hours. Unreacted doxorubicin was removed by extraction with pH 5.0 phosphate buffer and the product recrystallised from methanol to give a red solid. TLC studies using normal phase silica gel plastic backed plates with a mobile phase of chloroform:methanol:triethylamine (70:10:1) showed a component (4 mg, 3.65 µmol, 45.65%) (Rf 0.65) significantly different from doxorubicin (Rf 0.46).

Analysis: Mpt : 181-184°C.

IR : 1612 cm^{-1}, 1587 cm^{-1}, 1282 cm^{-1}, 1204 cm^{-1}, 1010 cm^{-1}, 990 cm^{-1} (Doxorubicin).

^1H NMR ($CDCl_3$) : (270 mHz) 0.91 (3H, t, CH_2CH_3), 1.17 (3H, d, CH3, C5'), 1.67 (2H, s, C2'), 1.90 (6H, s, NMe_2), 2.05 (2H, s, C9'), 2.17 (2H, s, $(CH_2)_8$), 2.49 (2H, s, C7), 2.29-2.81 (2H, m, $(CH_2)_8$), 2.78 (2H, t, OCH_2CH_2N), 2.51 (2H, t, CH_2CH_3), 3.20 (1H, s, C3'), 3.76 (1H, q, C5'), 3.54 (1H, s, C4'), 3.84 (1H, q, C5'), 3.85 (2H, q, OCH_2CH_2N), 4.19 (2H, s, C14), 4.53 (1H, s, C10), 5.63 (1H, s, C1'), 6.69 (2H, d, meta to OCH_2CH_2N), 6.78 (1H, t, C3), 6.82-7.31 remaining H,

(underlining indicates signals due to doxorubicin).

Mass Spectrum: m/e (relative intensity) 1040 ($C_{60}H_{66}NO_{15}$,67), 697 ($C_{36}H_{43}NO_{13}$,33).

Elemental Analysis: Found C, 68.10%; H, 6.41%; N, 2.21%, calculated for $C_{63}H_{72}N_2O_{15}$ C, 68.90%; H, 6.56%; N, 2.55%.

III. SYNTHESIS OF Z-4-HYDROXYTAMOXIFEN DODECANEDIOYL ESTER - DOXORUBICIN PRODRUG (DOXOTAM 12)

Z-4-Hydroxytamoxifen dodecanedioyl ester (Tam 12, 5 mg, 4.3 µmol) was dissolved in dichloromethane (10 ml). Doxorubicin HCl (5 mg, 8 µmol) was added with N,N' dicyclohexylcarbodiimide (10 mg, 48 µmol). The mixture was stirred at room temperature for 4 hours.

Unreacted doxorubicin was removed by extraction with pH 5.0 phosphate buffer and the product recrystallised from methanol to give a red solid. TLC studies using normal phase silica gel plastic backed plates with a mobile phase of chloroform:methanol: triethylamine (70:10:1) showed a component (3 mg, 2.60 µmol, 32.5%) (Rf 0.56) significantly different from doxorubicin (Rf 0.46).

Analysis: Mpt : 203-204°C.

IR : 1612 cm^{-1}, 1587 cm^{-1}, 1282 cm^{-1}, 1204 cm^{-1}, 1010 cm^{-1}, 990 cm^{-1} (Doxorubicin).

^1H NMR ($CDCl_3$) : (270 mHz) 0.91 (3H, t, CH_2CH_3), 1.22 (3H, d, C5'), 1.59 (2H, s, C2'), 2.22 (2H, s, C9), 2.29 (6H, s, NMe_2), 2.36 (2H, s, $(CH_2)_{12}$), 2.45 (2H, t, CH_2CH_3), 2.56 (2H, s, C7), 2.65 (2H, t, OCH_2CH_2N), 1.90-2.86 (2H, m, $(CH_2)_{12}$), 3.19 (1H, s, C3'), 3.46 (1H, s, C4'), 3.61 (3H, s, CH_3O), 3.75 (1H, q, C5'), 3.93 (2H, q, OCH_2CH_2N), 4.08 (2H, s, C14), 4.88 (1H, s, C10), 5.29 (1H, s, C1'), 6.58 (2H, d, meta to OCH_2CH_2N), 6.76 (1H, t, C3), 6.85-7.26 remaining H, (underlining indicates signals due to doxorubicin).

Mass Spectrum: m/e (relative intensity) 1098 ($C_{64}H_{76}NO_{15}$,33), 755 ($C_{40}H_{53}NO_{13}$,43).

Elemental Analysis: Found C, 70.51%; H, 7.29%; N, 3.51%, calculated for $C_{67}H_{80}N_2O_{15}$ C, 69.70%; H, 6.93%; N, 2.43%.

EXAMPLE 3

In vitro testing

The compounds prepared in Examples 1 and 2 above were tested in in vitro culture assays on an oestrogen receptor positive cell line MCF-7 (Soule, H.D. et al. (1973) J. Natl. Cancer Inst., 51, 1409-1416) and an oestrogen receptor negative cell line HS05787 (Hackett, A.J. et al. (1977) J. Natl. Cancer Inst., 58, 1795-1806). These were performed alongside controls of doxorubicin HCl and 4-hydroxytamoxifen alone and in combination.

The cell lines MCF-7 and HS-0578T were routinely maintained as monolayer cultures in RPMI 1640 (Gibco) cell culture medium supplemented with foetal calf serum (10%), sodium pyruvate (1 mM) penicillin/streptomycin (50 I.U. ml^{-1} of each), and buffered with HEPES (25 mM). The cells were incubated at 37°C in an atmosphere of 95% air/5% carbon dioxide.

Cytotoxicity studies

Cells were harvested from exponentially growing cultures via trypsinisation, counted with a haemocytometer, and diluted to produce appropriate suspensions. An aliquot of these cells (5×10^3 cells) were plated into each of 96 wells of a microtitre plate and incubated for 24 hours to ensure logarithmic growth. The cells were exposed for 96 hours to drug concentrations in the range 0.002 to 25.000 µM, and cytotoxicity was assessed by means of an MTT assay (Jabbar, S.A.B. et al., Br. J. Cancer, 60, 523-528). The results were expressed as IC_{50} values with respect to untreated control cells.

RESULTS

Table 1 below shows the IC_{50} (μM) values obtained for the compounds tested

Table 1

IC50 μM	ER^+ MCF-7	ER^- HS0578T
Doxorubicin	0.173	0.220
4-Hydroxytamoxifen	0.218	NA
DoxoTam 12	1.760	>25
DoxoTam 8	17.88	NA
DoxoTam 2	0.252	0.365
Tam 12	NA	NA
Tam 8	NA	NA
Tam 2	3.532	NA
Doxo 12	0.136	0.198
Doxo 8	0.303	0.370
Doxo 2	0.224	0.188

NA = Not Applicable

These results show that the prodrugs doxotam 12 and doxotam 8 were selectively toxic to the ER^+ cells whereas these prodrugs were inactive against the ER negative cell lines tested. Doxotam 2, on the other hand was unselective in its toxicity to both the MCF-7 and HS0578T cell lines, hence supporting the invention that the linker must be at a minimum length.

EXAMPLE 4

In vivo activity of Doxotam 12 in female nude Balb/C mice implanted with MCF-7 tumour cells.

Studies on the in vivo effects of Doxotam 12 have initially shown that this prodrug is remarkably non-toxic to rodents. The LD_{50} value for doxorubicin is 10 mg kg^{-1} by intravenous route in mice, hence initial toxicity studies with Balb/C nude mice (mean weight 30g, three mice per dose level) used escalating doses of Doxotam 12 by intravenous route up to a maximum dose of 20 mg kg^{-1} (since this contains the equivalent of 10 mg kg^{-1} doxurubicin). The mice suffered no apparent toxic effects from the highest dose administered, hence a new escalating regime was used up to 400 mg kg^{-1} were well tolerated by the mice.

In a parallel study, attempts were made to implant MCF-7 breast tumour spheroid cells by subcutaneous route into the right flank of immunodeficient female Balb/C nude mice. Initial attempts to implant tumour were unsuccessful, and it was suspected that these oestrogen receptor (ER) positive cells require oestrogen supplemention for growth. When subcutaneous oestradiol pellets were used, MCF-7 tumour growth in Balb/C mice was achieved. This enabled a study on the effects of Doxotam 12 on MCF-7 tumour bearing mice to be made.

Eleven Balb/C (30g) female nude mice were subcutaneously implanted with pellets containing 17β-oestradiol (0.72 mg, Innovative Research of America). A suspension containing spheroidal clumps of cultured MCF-7 cells (10^6 ml^{-1}) were innoculated into the right flank of these mice. The mice were left for a sufficient time period (38 days) for a tumour mass to be detected. The tumour mass was assessed by weekly caliper measurements, and the values for the relative tumour volume recorded (compared to volume at first detection). The mice were divided into two groups: in the first group six mice were used as controls, and were intravenously administered via the tail vein with the solvent used for prodrug solution (10% dimethyl sulphoxide in 0.9% NaCl); in the second group five animals were treated with

prodrug in solvent, and were administered with a single dose of 20 mg kg^{-1} Doxotam 12 by the intravenous route in the tail vein. Both groups were then monitored for relative tumour volume for up to 65 days, after which the mice were killed owing to the distress caused to the mice by the tumour. The results are shown in Table 2 below

Relative tumour volumes for implanted MCF-7 cells in female Balb/C nude mice

TABLE 2

Day	Solvent Control (n=6)	Doxotam12 20mg/kg i.v. (n=5)
0	1.00	1.00
7	1.70±0.18	1.61±0.54
14	2.04±0.57	1.47±1.34
21	3.58±1.49	2.23±1.44
31	6.42±2.80	3.89±3.13
38	8.91±3.76	4.75±3.48
44	10.80±3.65	6.31±4.93
51	13.60±4.77	9.19±5.50
58	18.20±5.57	11.20±6.37
65	24.80±9.82	13.40±7.43

These data show that the implanted tumours from the control group undergo an exponential, though relatively slow growth during the course of the experimental period. The drug treated group show an initial reduction in relative tumour volume, then exponential growth from 14 days onward. However there is no significant difference between pairs of equivalent points for these curves at the dosage level used.

These data indicate that Doxotam 12 has tumoricidal activity at 20 mg kg^{-1} via the intravenous route. These initial studies have shown that this tumoricidal activity has been achieved with little if any toxicity to the mice.

CLAIMS

1. Compounds comprising an antioestrogenic moiety linked to an antitumour moiety by means of a spacer group characterised in that the spacer group is a minimum of 10 atoms in length excluding any atoms from the antioestrogenic moiety or the antitumour moiety which contribute to the linkage.

2. Compounds as claimed in claim 1 in which the spacer group is from 10 to 22 or 24 atoms in length.

3. Compounds as claimed in claim 1 in which the spacer group is from 12 to 18 atoms in length.

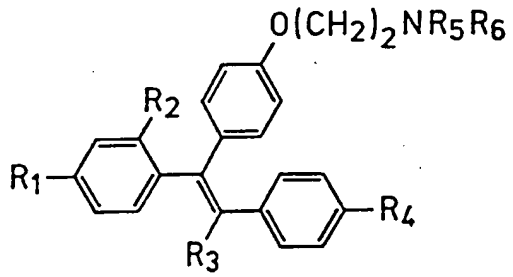
4. Compounds as claimed in claim 1 in which the spacer group is 14 atoms in length.

5. Compounds as claimed in claims 1, 2, 3 or 4 wherein the spacer group is a dicarboxylic acid, a peptide or a polysaccharride.

6. Compounds as claimed in claim 1 or 2 wherein the spacer group is a dicarboxylic acid of the formula $HOOC(CH_2)_nCOOH$ where n is from 8 to 20.

7. Compounds as claimed in any of claims 1 to 6, wherein the spacer group makes an ester or amide bond linkage with the antioestrogenic and antitumour moieties.

8. Compounds as claimed in claim 1, wherein the antioestrogenic moiety is selected from the group consisting of compounds of general formula (I)



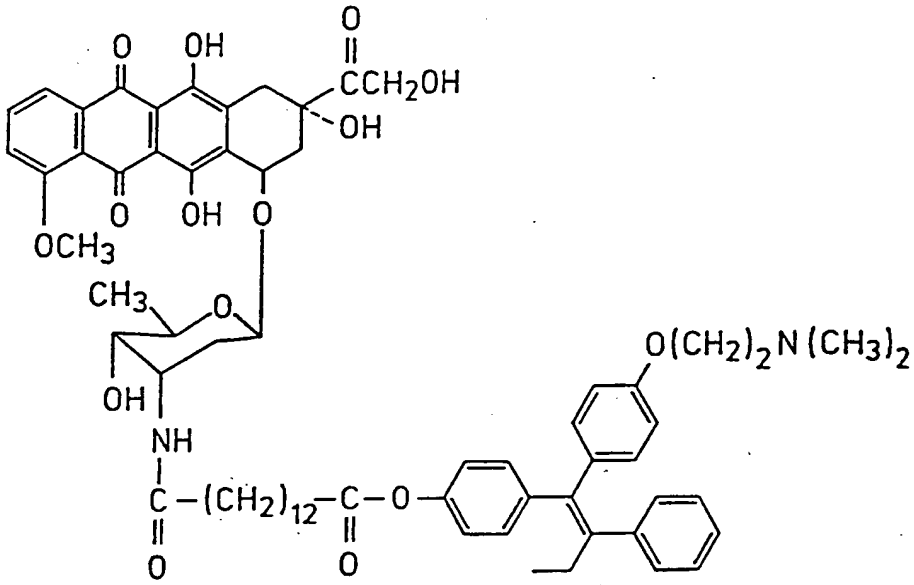
(I)

wherein R_1 is hydrogen or hydroxy, R_2 is hydrogen or C_{1-4} alkyl, R_3 is unsubstituted aliphatic C_{1-4} alkyl or aliphatic C_{1-4} alkyl

substituted by one or more halogen, nitro, amino, aldehyde, keto, hydrazino or alcohol groups, R_5 and R_6 being the same or different, or a 2,3-diphenylindole, 2-benzoyl-3-phenylthiophene, an acetoxy-substituted triarylethen, a 1,2-diphenylethane or a 2-phenylindene and the antitumour moiety is selected from the group consisting of compounds doxorubicin, methotrexate, mitoxantrone, danurobicin, epirubicin, idarubicin, acracinomycin A, DUP-941, cyclophosphamide or fosphamide.

9. Compounds as claimed in claim 8, wherein the antioestrogenic moiety is tamoxifen or 4-hydroxytamoxifen.

10. Compounds as claimed in any preceding claim that are of the formula (II)



(II)

11. Compounds that are a biological breakdown product of any of the compounds defined in any of claims 1 to 7, with the proviso that the breakdown product includes at least part of the spacer group.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/GB 94/01185

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07H15/252 A61K31/70 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07H C07C C07J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,90 10638 (EISENBRAND G.) 20 September 1990 see page 3, line 15 - page 6, line 13	1-11
X	WO,A,92 14493 (CAPELLI C.) 3 September 1992 see page 5, line 29 - page 7, line 32	1-11
A	GB,A,2 201 419 (FARMITALIA CARLO ERBA S.P.A.) 1 September 1988 see page 5 - page 6	1
A	US,A,5 149 794 (YATVIN M.B. ET AL) 22 September 1992 see column 3, line 10 - column 4, line 20	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search	Date of mailing of the international search report
30 August 1994	05. 09. 94

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/GB 94/01185

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9010638	20-09-90	DE-A-	3907290	13-09-90
WO-A-9214493	03-09-92	AU-A-	1424492	15-09-92
GB-A-2201419	01-09-88	NONE		
US-A-5149794	22-09-92	US-A-	5256641	26-10-93